THE PRODUCTION, THROUGH IMMUNIZATION, OF SPECIFIC FERMENTS AGAINST BACTERIA AS DETECTED BY THE ABDERHALDEN TEST*

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Since the appearance of Abderhalden's first papers concerning the principles and procedure involved in the serodiagnosis of pregnancy a voluminous literature has appeared. A great variety of physiological and pathological conditions have been investigated with discordant results in many cases. This fact is of itself significant in that it serves to emphasize the delicacy of the reaction, the many sources of error to which the worker is liable, and the importance of a uniform and highly perfected technic. In this connection it is noteworthy that many investigators have absolutely reversed their first opinions, as their skill in performing the test has increased.

The principle, as enunciated by Abderhalden, presupposes the production of a specific proteolytic ferment, elaborated in response to the parenteral introduction of a foreign protein.

That such a specificity of ferment production obtains in the case of tissue protein has been demonstrated conclusively in the opinion of many observers.[1] It must be admitted, however, that this opinion is not universal, in fact, there are those[2] who assert that no specificity exists. Others[3] admit specificity in the case of most organ preparations but affirm non-specificity in the case of

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1. Abderhalden: München. med. Wchnschr., 1914, 61, p. 401.
   Bruck: Ibid., 1913, 60, p. 1775.
   Fischer: Deutsch. med. Wchnschr., 1913, 39, p. 2138.
   Fuchs: München. med. Wchnschr., 1913, 60, p. 2230.
   v. Gambaroff: Ibid., p. 1644.
   Grey: Bull. Johns Hopkins Hosp., 1914, 25, p. 117.
   Hirsch: Deutsch. med. Wchnschr., 1914, 40, p. 270.
   Lampé: München. med. Wchnschr., 1914, 61, p. 463.
   Lampé and Fuchs: Deutsch. med. Wchnschr., 1914, 40, p. 747.
   Lampé and Papazolu: München. med. Wchnschr., 1913, 60, p. 1533.
   Lowy: Jour. Am. Med. Assn., 1914, 62, p. 437.
   Mayer: München. med. Wchnschr., 1913, 60, p. 2906.
   Schiff: Ibid., 1914, 61, p. 768.
   Veit: Berl. klin. Wchnschr., 1913, 50, p. 1241.
   Wegener: München. med. Wchnschr., 1914, 61, p. 15.
   Williamson: Jour. Obst. and Gynec. Brit. Emp., 1913, 24, p. 211.
2. Heilner and Petri: München. med. Wchnschr., 1913, 60, p. 1530.
   Lange: Berl. klin. Wchnschr., 1914, 51, p. 785; Biochem. Ztschr., 1914, 61, p. 193.
   Michaelis and v. Lagermarck: Deutsch. med. Wchnschr., 1914, 40, p. 316.
   Mosbacher and Port: Ibid., p. 1410.
   Oeller and Stephan: München. med. Wchnschr., 1914, 61, pp. 75, 579; Deutsch. med. Wchnschr., 1914, 40, p. 1557.
3. Bauer: Med. Klin., 1913, 9, p. 1797.
   Frank, Rosenthal, and Biberstein: München. med. Wchnschr., 1913, 60, p. 1594.

some tissues, such as kidney tissue. Finally, there are those[4] who maintain vigorously their belief in group reactions.

With respect to the application of the test, by far the greatest amount of work has been done on pregnancy, cancer, and mental disorders. From a review of the literature it would appear that its value as a diagnostic procedure in such cases is generally accepted.

With respect to the application of the reaction as a diagnostic method in infectious diseases, tuberculosis[5] has received the greatest amount of attention. In this connection it must be admitted that the majority of workers report results far from satisfactory. In a few cases, however, the reports[6] have been most encouraging, and in the opinion of some investigators tuberculosis in its various forms, degrees of severity, and duration can be differentiated by means of the nature of the substrate degraded. The sera from typhoid patients have been shown to contain a ferment capable of digesting typhoid bacilli.[7] Reactions in the case of syphilitic sera[8] are apparently specific.

Thus far, but one paper[9] has appeared in which the limits of specificity of bacterial ferments have been considered. According to this report, the authors have demonstrated ferments which to a certain extent were specific, in that group reactions were obtained. The ferment produced by the inoculation of typhoid organisms was capable of degrading not only typhoid but also paratyphoid and the colon bacilli. Usually, such distantly related organisms as the typhoid bacillus and the staphylococcus could be differentiated, but even here these authors did not obtain uniformly specific results.

The experiment to be reported in this paper was designed to test the following hypothesis: The protein portions of bacteria which are capable of producing immunity, that is, the haptophorous groups of Vaughan, are essentially different in molecular constitution or configuration and when introduced parenterally, as in immunization, call forth ferments which are specific for the particular haptophore introduced.

In carrying out the work, the organisms employed were Sta. aureus, Sta. albus, streptococcus, pneumococcus, B. influenzae, and M. catarrhalis. These particular organisms were selected because of their application to another problem which was under consideration, yet

4. Kotchneff and Chingarewa: Compt. rend. Soc. de biol., 1914, 76, p. 354.
Schingarewa and Kotschnëwa: Russk. Vrach., 1914, 13, p. 101.
Singer: München.. med. Wchnschr., 1914, 61, p. 350.
5. Fränkel and Gumpertz: Deutsch. med. Wchnschr., 1913, 39, p. 1585.
Gumpertz: Beitr. z. Klin. d. Tuberk., 1914, 30, p. 201.
Jessen: Ibid, 1913, 28, p. 489.
Krim: Russk. Vrach., 1913, 12, p. 1502.
Lampé: Deutsch. med. Wchnschr., 1913, 39, p. 1774.
Melikjanz: Deutsch. med. Wchnschr., 1914, 40, p. 1369.
6. Fränkel: Deutsch. med. Wchnschr., 1914, 40, p. 589.
Gwerder and Melikjanz: München. med. Wchnschr., 1914, 61, p. 980.
Jessen: Beitr. z. Klin. d. Tuberk., 1913, 28, p. 489.
Lampé: Deutsch. med. Wchnschr., 1913, 39, p. 1774.
Wolff and Frank: Berl. klin. Wchnschr., 1914, 51, p. 875.
7. Voelkel: München. med. Wchnschr., 1911, 61, p. 349.
8. Reines: Wien. med. Wchnschr., 1914, 64, p. 368.
Varney and Morse: Jour. Michigan Med. Soc., 1914, 13, p. 515.
Voelkel: München. med. Wchnschr., 1914, 61, p. 349.
9. Fekete and Gàl: Monatschr. f. Geburtsh. u. Gynäk., 1914, 39, p. 21.

it was felt that they would adapt themselves to this work since some of them are closely enough related to test effectively a comparatively high degree of specificity, while others are so distantly related that should only group reactions obtain, these also could be detected.

Suspensions of the organisms were prepared for use as vaccines in the production of immune sera in rabbits. The organisms were cultivated on either plain or blood agar. The growth was removed and was washed three times in physiological saline solution by means of the centrifuge. Bacterial counts of the resultant emulsions were made and the desired dilutions prepared. These were then held for one hour at temperatures ranging from 56 to 60 C.

The dilutions were as follows: Pneumococcus, streptococcus, B. influenzae, and M. catarrhalis, 12.5, 25, 50, 100, and 200 millions; of Sta. aureus and Sta. albus, 50, 100, 200, 400, and 800 millions. A series of mixtures containing each of the organisms in the same number as used for the individual dilutions was also prepared.

These dilutions were injected intravenously at intervals of four days. The animals used were apparently normal rabbits of both sexes and of approximately the same weight. Two rabbits received the immunizing course with each individual organism, and two with the mixture.

During the administration of the immunizing course, the bacteria, which were to serve as substrates, were grown. Agar bottles were inoculated with each of the organisms and, after a suitable incubation, the growth was removed by washing, was filtered through fine silk, and collected in centrifuge tubes. The process of washing by centrifugalization was repeated from three to seven times; those organisms which could be cultivated on plain agar received only three washings in physiological saline solution, while those which had to be grown on blood agar received a greater number. In the case of this last type, alternate washings in saline and water were given and, in this way, apparently all of the hemoglobin contained in the blood corpuscles, which were washed off with the bacterial growth, was removed. After the last washing a thick white emulsion was obtained, which was killed by heat as in the case of the vaccine preparations. The emulsions were then centrifugalized again, the supernatant fluid drawn off, and the bacterial residue placed in vacuum jars over sulphuric acid and dried. After through drying, the bacteria were pulverized as finely as possible and kept in the ice-box.

The dialyzing thimbles (Schleicher and Schüll No. 579A) were tested for impermeability with normal horse or rabbit serum. Those giving any trace of blue color when the dialysate was tested were discarded. The thimbles were also tested for impermeability against the staphylococcus aureus, but in no case was a positive reaction obtained where serum failed to give a positive result. Silk-peptone was used in testing for permeability. After the dialyzing thimbles had been tested they were kept in chloroform water under a layer of toluene and immediately before use they were boiled in several changes of distilled water. All of the glassware, corks, etc., were sterilized before use.

For purposes of testing, the animals were divided into two groups; one of the two rabbits receiving each kind of organism and one of those receiving the mixture being placed in each group. The rabbits of the first group were tested for ferment production about one week after the completion of the immunizing course. Those of the second group were tested about two weeks after the last immunizing injection. In obtaining the serum the animals were anesthetized,

the carotid artery exposed, and the blood collected in large tubes. Sterile precautions were observed throughout. As has been advocated by many observers,[10] all food was denied the animals for at least eighteen hours before the withdrawal of blood.

When the serum had separated from the clot, it was drawn off and centrifugalized to free it from all cellular elements and then held in the ice-box until used. In no case did an interval greater than five hours elapse between the bleeding and the use of the serum. The control sera were obtained in a similar manner as the test sera, and fresh serum was used for control purposes with each day's tests.

In performing the test, 1.5 c.c. of serum and 10 mg. of the dried bacterial substrate were always used. The quantity of substrate was chosen arbitrarily but, since it was found that 10 mg. gave a very satisfactory result with positive sera, this amount was always used, altho probably a smaller quantity would have sufficed. Each serum under test was combined with the homologous substrate and also with the substrates prepared from each of the other organisms used in the experiment. The serum and substrate were now dialyzed against 20 c.c. of sterile, distilled water. Toluene was added both to the contents of the thimble and to the distilled water in the outside container.

Digestion and dialysis were allowed to proceed for sixteen hours at 37 C.

For control purposes the serum was dialyzed alone; the specific substrate alone, suspended in physiological salt solution; the substrate combined with normal serum; and normal serum alone.

At the completion of the period of dialysis 10 c.c. of the dialysate were removed, 0.2 c.c. of a 1 percent solution of ninhydrin was added, and the whole boiled for exactly one minute. Readings were taken at the end of one-half hour.

The results are summarized in the accompanying table.

DISCUSSION OF RESULTS

From the table it is evident that each of the sera, when combined with its homologous substrate, gave a very definite positive reaction. Catarrhalis sera reacted positively when combined with catarrhalis, and also in one serum slight reactions were obtained when streptococcus and the staphylococcus albus were used as substrates. Identical results were obtained with influenza serum. Serum 818 degraded influenza alone. Pneumococcus and streptococcus sera were specific, degrading none but the homologous substrates. One of the staphylococcus aureus sera gave a weakly positive reaction with streptococcus substrate. Staphylococcus albus degraded albus only.

10. Abderhalden and Lampé: Ztschr. f. physiol. Chem., 1913, 85, p. 136.
Ball: Jour. Am. Med. Assn., 1914, 62, p. 599; Ibid., 63, p. 1169.
Grey: Bull. Johns Hopkins Hosp., 1914, 25, p. 117.
Lowy: Jour. Am. Med. Assn., 1914, 62, p. 437.
Scherer: Berl. klin. Wchnschr., 1913, 50 p. 2183.
Schulz: München. med. Wchnschr., 1913, 60, p. 2512.

TABLE 1

Serum			Date of Last Injection	Date Serum Was Tested	Substrates						
					Catarrhalis	Influenza	Pneumococcus	Streptococcus	Sta. Aureus	Sta. Albus	None
Catarrhalis	815	♀	April 22	April 29	++++	0	0	+	0	+	0
	816	♂	April 22	May 6	++++	0	0	0	0	0	0
Influenza	817	♀	April 22	May 1	0	++++	0	+	0	+	0
	818	♂	April 22	May 6	0	++++	0	0	0	0	0
Pneumococcus	819	♂	April 24	May 2	0	0	++++	0	0	0	0
	820	♀	April 24	May 7	0	0	++++	0	0	0	0
Streptococcus	821	♂	April 24	May 2	0	0	0	++++	0	0	0
	822	♂	April 24	May 7	0	0	0	++++	0	0	0
Sta. aureus	823	♀	April 26	May 3	0	0	0	0	++++	0	0
	824	♂	April 26	May 12	0	0	0	+	++++	0	0
Sta. albus	825	♀	April 26	May 3	0	0	0	0	0	++++	0
	826	♀	April 26	May 11	0	0	0	0	0	++++	0
Mixture	827	♀	April 26	May 4	++++	++++	++++	++++	++++	++++	0
	828	♀	April 26	May 11	++++	++++	++++	++++	++++	++++	0
Normal	...	..	.....	.....	0	0	0	0	0	0	0
	..	..	.....	.....	0	0	0	0	0	0	0
None	...	..	.....	.....	0	0	0	0	0	0	

In the case of the rabbits, which received the immunizing course with the mixture, positive reactions were obtained with each of the different organisms. The controls in every case were perfect.

To explain the apparently non-specific results obtained in the combinations, catarrhalis-streptococcus and catarrhalis-staphylococcus albus, influenza-streptococcus and influenza-staphylococcus albus, and staphylococcus aureus-streptococcus, it may be said that in a subsequent experiment thirteen apparently normal rabbits have been tested for natural ferments against various strains of streptococci. In five of these rabbits positive reactions were obtained. This indicates that ferments which are capable of splitting streptococci, are present, perhaps as the result of accidental infection.

CONCLUSION

The only conclusion to be made at this time, disregarding entirely the theoretical aspects of the work, is that as the result of immunization ferments are produced which are specific for the organisms employed in the immunizing treatment.